

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/26070 <b>(22) International Filing Date:</b> 9 December 1998 (09.12.98) <b>(30) Priority Data:</b> 60/069,208 11 December 1997 (11.12.97) US <b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> DEL VECCHIO, Alfred [US/US]; 668 Matro Court, West Chester, PA 19103 (US). <b>(74) Agents:</b> HECHT, Elizabeth, J. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).		<b>(81) Designated States:</b> AL, AU, BA, BB, BG, BR, CA, CN, CZ, EE, GE, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HEPATITIS C VIRUS NS5B TRUNCATED PROTEIN AND METHODS THEREOF TO IDENTIFY ANTIVIRAL COMPOUNDS		
<b>(57) Abstract</b>  The invention provides HCV NS5B polypeptides and DNA (RNA) encoding HCV NS5B polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing HCV NS5B polypeptides to screen for antiviral compounds.		

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## HEPATITIS C VIRUS NS5B TRUNCATED PROTEIN AND METHODS THEREOF TO IDENTIFY ANTIVIRAL COMPOUNDS

### CROSS-REFERENCE TO RELATED APPLICATION

- 5           This application claims benefit to the earlier provisional U.S. application, Serial No. 60/069,208, filed on December 11, 1997, the contents of which are incorporated herein by reference in their entirety.

### FIELD OF THE INVENTION

- 10           This invention relates to newly identified polynucleotides and polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, in these and in other regards, the invention relates to novel polynucleotides and polypeptides of viruses of the *Flaviviridae* family, particularly a novel truncate of the Hepatitis C Virus (HCV)  
15   NS5B protein, as well as other variants disclosed herein, all hereinafter referred to as "HCV NS5B".

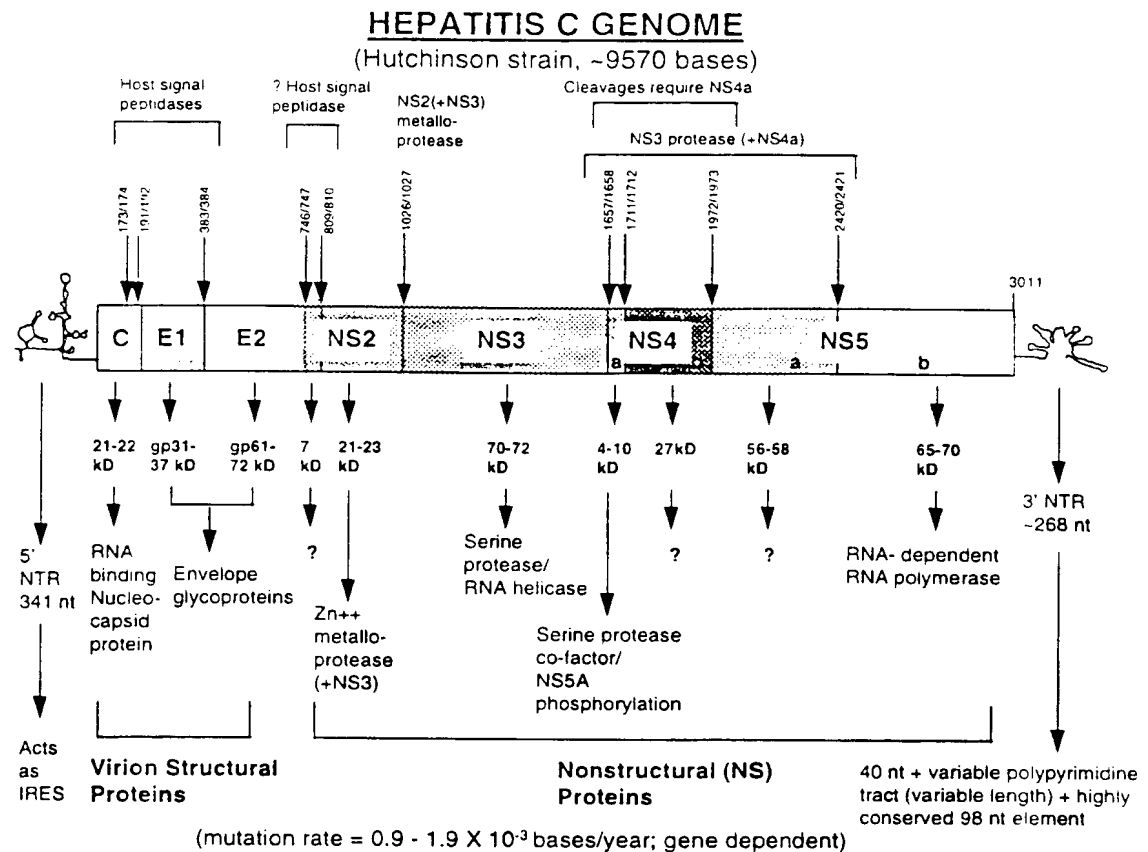
### BACKGROUND OF THE INVENTION

- First identified by molecular cloning in 1989 (Choo, *et al.*, *Science* 244:  
20   359-362 (1989)), hepatitis C virus (HCV) is now widely accepted as the most common causative agent of post-transfusion non A, non-B hepatitis (NANBH) (Kuo, *et al.*, *Science* 244:362-364 (1989)). Infection with HCV is a major cause of human liver disease throughout the world with seroprevalence in the general population ranging from 0.3 to 2.2% (van der Poel, *et al.*, HEPATITIS C VIRUS; Amsterdam:Karger; pp. 137-163 (1994)) to as high as ~10-20% in Egypt (Hibbs,  
25   *et al.*, *J. Infect. Dis.* 168: 789-790 (1993)). Although the virus is most commonly transmitted via blood, the mode of transmission remains unknown for a large portion of infected individuals (Alter, M.J., *Infect. Agents Dis.* 2: 155-166 (1993)). Over 50% of infections by HCV progress to acute hepatitis associated with  
30   viremia and generally elevated serum alanine aminotransferase (ALT) levels (Alter, H. J., CURRENT PROSPECTIVES IN HEPATOLOGY; New York:Plenum; pp. 83-97 (1989)). Over half of the acute cases progress to a chronic infection with roughly 20% developing cirrhosis (Alter, H. J., *supra*).

Chronic infection by HCV has also been linked epidemiologically to the development of hepatocellular carcinoma (HCC), especially in cirrhotic patients (Blum, *et al.*, *Hepatology* 19: 251-258 (1994)).

Since its initial identification, many isolates of HCV have been sequenced, displaying much genetic diversity and leading to the subclassification of this virus into multiple genotypes. See, e.g., Bukh, *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8234-8238 (1993); Bukh, *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 8239-8243 (1994); Dusheiko, *et al.*, *Hepatology* 19: 13-18 (1994)). Due to its genome structure and sequence homology, this virus was assigned as a new genus in the *Flaviviridae* family, along with the other two genera, flaviviruses (such as yellow fever virus and Dengue virus types 1-4) and pestiviruses (such as bovine viral diarrhea virus). See, e.g., Choo, *et al.*, *supra*; Miller, *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 2057-2061 (1990)). Like the other members of the *Flaviviridae* family, HCV is an enveloped virus containing a single-stranded RNA molecule of positive polarity. The HCV genome is approximately 9.6 kilobases (kb) with a long, highly conserved, noncapped 5' nontranslated region (NTR) of approximately 340 bases which functions as an internal ribosome entry site (IRES) (Wang, *et al.*, *Curr. Topics Microbiol. Immunol.* 203: 99-112 (1995)). This element is followed by a region which encodes a single long open reading frame (ORF) encoding a polypeptide of ~3000 amino acids comprising both the structural and nonstructural viral proteins. This large polypeptide is subsequently processed into the individual structural and nonstructural proteins by a combination of host and virally-encoded proteinases (reviewed in Rice, *VIROLOGY*; Raven Press:New York, 2nd Ed.; pp. 931-960 (1996)). Table 1 is a diagram of the viral proteins of HCV. In their respective order in the polyprotein precursor from amino-terminus to carboxy-terminus, are core-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B.

Table 1



### Inhibition of the biological activity of viral proteins of the *Flaviviridae*

- 5 family, particularly HCV NS5B, is potentially of benefit in controlling, reducing and alleviating the diseases caused by infection with these viral organisms. Clearly, there is a need for factors, such as the novel compounds of the invention, that have a present benefit of being useful to screen compounds for antiviral activity. Such factors are also useful in determining their role in pathogenesis of
- 10 infection, dysfunction and disease. There is also a need for identification and characterization of such factors and their antagonists and agonists which can play a role in preventing, ameliorating or correcting infections, dysfunctions or diseases.

- In an attempt to identify the critical domains of the HCV NS5B protein,
- 15 Lohmann, *et al.*, *J. Virol.* 8416-8428 (1997) truncated both the amino and carboxy termini of the full-length HCV NS5B protein. While Lohmann, *et al.* discuss various sequence fragments of the HCV NS5B protein, with one exception of a known GenBank

sequence, the reference does not specifically disclose either the cDNA or amino acid sequences of these fragments.

Clearly, there is a need to discover new antiviral compounds that are useful in the cure, treatment, and prevention of virus infection of the *Flaviviridae* family,

- 5 particularly HCV. Because the HCV NS5B protein is difficult to express, it is important to discover forms of the protein which may be more soluble for use in a drug-discovery screen than is the full-length HCV NS5B protein. The instant invention is believed to provide for such a need in the antiviral area.

## 10 SUMMARY OF THE INVENTION

It is an object of the invention to provide polypeptides that have been identified as novel HCV NS5B polypeptides by homology between the amino acid sequence set forth in Table 2 [SEQ ID NO:2] and other known HCV amino acid sequences, such as those shown in Table 4.

- 15 It is a further object of the invention to provide polynucleotides that encode these novel HCV NS5B polypeptides, particularly polynucleotides that encode the polypeptide, herein designated HCV NS5B.

- In a particularly preferred embodiment of the invention, there is provided a polynucleotide which comprises a region encoding the HCV NS5B polypeptides set forth in Table 3 [SEQ ID NO:3] and which includes, for example, one or more variants thereof.

In another particularly preferred embodiment of the invention, there is a novel truncation mutant of the HCV NS5B protein comprising the amino acid sequence set forth in Table 3 [SEQ ID NO:4], or one or more variants thereof.

- 25 In a further aspect of the invention there are provided isolated nucleic acid molecules encoding HCV NS5B, including mRNAs, cDNAs, genomic DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

- 30 In accordance with another aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization. Among the particularly preferred

embodiments of the invention are naturally occurring allelic variants of HCV NS5B polypeptides.

Another aspect of the invention provides novel polypeptides of HCV NS5B, as well as biologically, diagnostically, prophylactically, clinically or  
5 therapeutically useful variants thereof, and compositions comprising the same.

Among the particularly preferred embodiments of the invention are variants of HCV NS5B polypeptide encoded by naturally occurring alleles of the HCV NS5B gene.

In a preferred embodiment of the invention, there are provided methods  
10 for producing the aforementioned HCV NS5B polypeptides.

In accordance with yet another aspect of the invention, there are provided for inhibitors to such polypeptides, useful as antiviral agents, including, for example, antibodies.

In accordance with certain preferred embodiments of the invention, there  
15 are provided products, compositions and methods for assessing HCV NS5B expression, treating disease, for example, viruses linked to the family, particularly HCV; flaviviruses such as yellow fever virus; Dengue virus types 1-4; and pestiviruses, such as bovine viral diarrhea virus and classic swine fever, among others, assaying genetic variation., and administering a HCV NS5B polypeptide  
20 or polynucleotide to an organism to raise an immunological response against a virus of the *Flaviviridae* family, especially HCV NS5B.

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided polynucleotides that hybridize to HCV NS5B polynucleotide sequences, particularly under stringent conditions.

25 In certain preferred embodiments of the invention there are provided antibodies against HCV NS5B polypeptides.

In other embodiments of the invention, there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit, or activate, an activity of a polypeptide or polynucleotide of the invention  
30 comprising: contacting a polypeptide or polynucleotide of the invention with a compound to be screened under conditions to permit binding to, or other interaction between, the compound and the polypeptide or polynucleotide to

assess the binding to or other interaction with the compound, such binding or interaction being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide or polynucleotide with the compound; and determining whether the compound binds  
5 to or otherwise interacts with and activates or inhibits an activity of the polypeptide or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the polypeptide or polynucleotide.

In accordance with yet another aspect of the invention, there are provided  
10 HCV NS5B agonists and antagonists, preferably virustatic agonists and antagonists.

In a further aspect of the invention there are provided compositions comprising an HCV NS5B polynucleotide or polypeptide for administration to a cell or to a multicellular organism.

15 Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

## 20 GLOSSARY

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Host cell" is a cell which has been transformed, transfected or infected, or is capable of transformation, transfection or infection by an exogenous  
25 polynucleotide sequence.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the  
30 case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (COMPUTATIONAL MOLECULAR



BIOLOGY, Lesk. A.M., ed., Oxford University Press, New York, 1988;  
BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W.,  
ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF  
SEQUENCE DATA, PART I. Griffin, A.M., and Griffin, H.G., eds., Humana  
5 Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje,  
G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and  
Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and  
Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to  
determine identity are designed to give the largest match between the sequences  
10 tested. Methods to determine identity and similarity are codified in publicly  
available computer programs. Preferred computer program methods to determine  
identity and similarity between two sequences include, but are not limited to, the  
GCG program package (Devereux, *et al.*, *Nucleic Acids Research* 12(1): 387  
(1984)), BLASTP, BLASTN, and FASTA (Atschul, *et al.*, *J. Molec. Biol.* 215:  
15 403-410 (1990). The BLAST X program is publicly available from NCBI and  
other sources (BLAST Manual, Altschul, S., *et al.*, NCBI NLM NIH Bethesda,  
MD 20894; Altschul, *et al.*, *J. Mol. Biol.* 215: 403-410 (1990).

As an illustration, by a polynucleotide having a nucleotide sequence  
having at least, for example, 95% "identity" to a reference nucleotide sequence of  
20 SEQ ID NO:3, it is intended that the nucleotide sequence of the polynucleotide is  
identical to the reference sequence, except that the polynucleotide sequence may  
include up to five point mutations per each 100 nucleotides of the reference  
nucleotide sequence of SEQ ID NO:3. In other words, to obtain a polynucleotide  
having a nucleotide sequence at least 95% identical to a reference nucleotide  
25 sequence, up to 5% of the nucleotides in the reference sequence may be deleted or  
substituted with another nucleotide, or a number of nucleotides up to 5% of the  
total nucleotides in the reference sequence may be inserted into the reference  
sequence. These mutations of the reference sequence may occur at the 5' or 3'  
terminal positions of the reference nucleotide sequence or anywhere between  
30 those terminal positions, interspersed either individually among nucleotides in the  
reference sequence or in one or more contiguous groups within the reference  
sequence. Analogously, by a polypeptide having an amino acid sequence having

at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:4, it is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO:4. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

"Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but

more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones  
5 modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many  
10 useful purposes known to those of skill in the art. The term "polynucleotide(s)", as it is employed herein, embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often  
15 referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins.  
20 Polypeptides may contain amino acids other than the 20 gene encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and  
25 they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl  
30 termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative,

covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor  
5 formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and  
10 ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., *Posttranslational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B.  
15 C. Johnson, Ed., Academic Press, New York (1983); Seifter, *et al.*, *Meth. Enzymol.* 182:626-646 (1990) and Rattan, *et al.*, *Protein Synthesis: Posttranslational Modifications and Aging*, *Ann. N.Y. Acad. Sci.* 663: 48-62 (1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-  
20 translational natural processes and may be made by entirely synthetic methods, as well.

"Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in  
25 nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed  
30 below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall

and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be  
 5 a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

## 10 DETAILED DESCRIPTION OF THE INVENTION

The full-length HCV NS5B nucleotide and amino acid sequences are set forth in Table 2 [SEQ ID NO:1 and SEQ ID NO:2, respectively].

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Table 2

### 15 Polynucleotide Sequence of HCV NS5B [SEQ ID NO:1]

TC AATGTCTTAT

20	11801	ACCTGGACAG	GCGCACTCGT	CACCCCGTGC	GCTGCGGAAG	AACAAAACT
	11851	GCCCATCAAC	GCACTGAGCA	ACTCGTTGCT	ACGCCATCAC	AATCTGGTAT
	11901	ATTCCACCAC	TTCACGCAGT	GCTTGCCAAA	GGCAGAAGAA	AGTCACATTT
25	11951	GACAGACTGC	AAGTTCTGGA	CAGCCATTAC	CAGGACGTGC	TCAAGGAGGT
	12001	CAAAGCAGCG	GCGTCAAAAG	TGAAGGCTAA	CTTGCTATCC	GTAAGGAAG
30	12051	CTTGCAGCCT	GACGCCCCCA	CATTGAGCCA	AATCCAAGTT	TGGCTATGGG
	12101	GCAAAAGACG	TCCGTTGCCA	TGCCAGAAAG	GCCGTAGCCC	ACATCAACTC
	12151	CGTGTGGAAA	GACCTTCTGG	AAGACAGTGT	AACACCAATA	GACACTATCA
35	12201	TCATGGCCAA	GAACGAGGTC	TTCTGCGTTC	AGCCTGAGAA	GGGGGGTCGT
	12251	AAGCCAGCTC	GTCTCATCGT	GTTCCCCGAC	CTGGGCGTGC	GCGTGTGCGA
	12301	GAAGATGGCC	CTGTACGACG	TGGTTAGCAA	ACTCCCCCTG	GCCGTGATGG
40	12351	GAAGCTCCTA	CGGATTCCAA	TACTCACCAG	GACAGCGGGT	TGAATTCCTC
	12401	GTGCAAGCGT	GGAAGTCCAA	GAAGACCCCG	ATGGGGTTCC	CGTATGATAC

12451 CCGCTGTTTT GACTCCACAG TCACTGAGAG CGACATCCGT ACGGAGGAGG  
12501 CAATTTACCA ATGTTGTGAC CTGGACCCCC AAGCCCGCGT GGCCATCAG  
5 12551 TCCCTCACTG AGAGGCTTTA TGTGCGGGGC CCTCTTACCA ATTCAAGGGG  
12601 GGAAACTGC GGCTATCGCA GGTGCCGCGC GAGCGGCGTA CTGACAACTA  
10 12651 GCTGTGGTAA CACCCTCACT TGCTACATCA AGGCCCGGGC AGCCCGTCGA  
12701 GCCGCAGGGC TCCAGGACTG CACCATGCTC GTGTGTGGCG ACGACTTAGT  
12751 CGTTATCTGT GAAAGTGCGG GGGTCCAGGA GGACGCGGCG AGCCTGAGAG  
15 12801 CCTTTACGGA GGCTATGACC AGGTACTCCG CCCCCCCCCG GGACCCCCCA  
12851 CAACCAGAAT ACGACTTGGA GCTTATAACA TCATGCTCCT CCAACGTGTC  
12901 AGTCGCCAC GACGGCGCTG GAAAAAGGGT CTACTACCTT ACCCGTGACC  
20 12951 CTACAACCCC CTCGCGAGA GCCGCGTGGG AGACAGCAAG ACACACTCCA  
13001 GTCAATTCCT GGCTAGGCAA CATAATCATG TTTGCCCCCA CACTGTGGGC  
25 13051 GAGGATGATA CTGATGACCC ATTTCTTTAG CGTCCTCATA GCCAGGGATC  
13101 AGCTTGAACA GGCTCTTAAC TGTGAGATCT ACGCAGCCTG CTACTCCATA  
13151 GAACCACTGG ATCTACCTCC AATCATTCAA AGACTCCATG GCCTCAGCGC  
30 13201 ATTTTACTC CACAGTTACT CTCCAGGTGA AGTCAATAGG GTGGCCGCAT  
13251 GCCTCAGAAA ACTTGGGGTC CCGCCCTTGC GAGCTTGAG ACACCGGGCC  
35 13301 CGGAGCGTCC GCGCTAGGCT TCTGTCCAGG GGAGGCAGGG CTGCCATATG  
13351 TGGCAAGTAC CTCTTCAACT GGGCAGTAAG AACAAAGCTC AACTCACTC  
13401 CAATAGCGGC CGCTGGCCGG CTGGACTTGT CCGGTTGGTT CACGGCTGGC  
40 13451 TACAGCGGGG GAGACATTTA TCACAGCGTG TCTCATGCCC GGCCCCGC

## Polypeptide Sequence of HCV NS5B [SEQ ID NO:2]

1 SMSYTWGAL VTPCAAEEQK LPINALSNSL LRHHNLVYST TSRSACQRQK  
 5 51 KVTFDRLQVL DSHYQDVLKE VKAAASKVKA NLLSVEEACS LTPPHSAKSK  
 101 FGYGAKDVRC HARKAVAHIN SVWKDLLEDV VTPIDTTIMA KNEVFCVQPE  
 151 KGGRKPARLI VFDPDLGVRVC EKMALYDVVS KLPLAVMGSS YGFQYSPGQR  
 10 201 VEFLVQAWKS KKTTPMGFSYD TRCFDSTVTE SDIRTEEAII QCCDLDPQAR  
 251 VAIKSLTERL YVGGPLTNSR GENCGYRRCR ASGVLTTSCG NTLTCYIKAR  
 15 301 AACRAAGLQD CTMLVCGDDL VVICESAGVQ EDAASLRAFT EAMTRYSAPP  
 351 GDPPQPEYDL ELITSCSSNV SVAHDGAGKR VYYLTRDPTT PLARAAWETA  
 401 RHTPVNSWLG NIIMFAPTLW ARMILMTHFF SVLIARDQLE QALNCEIYGA  
 20 451 CYSIEPLDLP PIIQRLHGLS AFSLHSYSPG EINRVAACLR KLGVPPLRAW  
 501 RHRARSVRAR LLSRGGRAAI CGKYLFWAV RTKLKLTPIP AAGRLDLSGW  
 25 551 FTAGYSGGDI YHSVSHARPR WFWFCLLLLA AGVGIYLLPN R

A particularly preferred embodiment of the invention relates to the HCV  
 NS5B truncation mutant having the nucleotide and amino acid sequences set out  
 in Table 3 [SEQ ID NO:3 and SEQ ID NO:4, respectively].

Table 3

## HCV NS5B Truncation Mutant Polynucleotide Sequence [SEQ ID NO:3]

35 1 TCAATGTCTT ATTCCTGGAC AGGCGCACTC GTCACCCCGT GCGCTGCGGA  
 51 AGAACAAAAA CTGCCCATCA ACGCACTGAG CAACTCGTTG CTACGCCATC  
 101 ACAATCTGGT GTATTCCACC ACTTCACGCA GTGCTTGCCA AAGGCAGAAG  
 40 151 AAAGTCACAT TTGACAGACT GCAAGTTCTG GACAGCCATT ACCAGGACGT  
 201 GCTCAAGGAG GTCAAAGCAG CGGCGTCAAA AGTGAAGGCT AACTTGCTAT  
 251 CCGTAGAGGA AGCTTGCAGC CTGACGCCCC CACATTCAGC CAAATCCAAG

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301 TTTGGCTATG GGGCAAAAGA CGTCCGTTGC CATGCCAGAA AGGCCGTAGC  
351 CCACATCAAC TCCGTGTGGA AAGACCTTCT GGAAGACAGT GTAACACCAA  
401 TAGACACTAC CATCATGGCC AAGAACGAGG TTTTCTGCGT TCAGCCTGAG  
451 AAGGGGGGTC GTAAGCCAGC TCGTCTCATC GTGTTCCCCG ACCTGGGCGT  
501 GCGCGTGTGC GAGAAGATGG CCCTGTACGA CGTGGTTAGC AAGCTCCCCC  
551 TGGCCGTGAT GGGGAAGCTCC TACGGATTCC AATACTCACC AGGACAGCGG  
601 GTTGAATTCC TCGTGCAAGC GTGGAAGTCC AAGAAGACCC CGATGGGGTT  
651 CTCGTATGAT ACCCGCTGTT TTGACTCCAC AGTCACTGAG AGCGACATCC  
701 GTACGGAGGA GGCAATTTAC CAATGTTGTG ACCTGGACCC CCAAGCCCCG  
751 GTGGCCATCA AGTCCCTCAC TGAGAGGCTT TATGTTGGGG GCCCTCTTAC  
801 CAATTCAAGG GGGGAAAAC T GCGGCTACCG CAGGTGCCGC GCGAGCGGCG  
851 TACTGACAAC TAGCTGTGGT AACACCCTCA CTGCTACAT CAAGGCCCGG  
901 GCAGCCTGTC GAGCCGCAGG GCTCCAGGAC TGCACCATGC TCGTGTGTGG  
951 CGACGACTTA GTCGTTATCT GTGAAAGTGC GGGGGTCCAG GAGGACGCGG  
1001 CGAGCCTGAG AGCCTTCACG GAGGCTATGA CCAGGTACTC CGCCCCCCCC  
1051 GGGGACCCCC CACAACCAGA ATACGACTTG GAGCTTATAA CATCATGCTC  
1101 CTCCAACGTG TCAGTCGCCC ACGACGGCGC TGGAAAGAGG GTCTACTACC  
1151 TTACCCGTGA CCCTACAACC CCCCTCGCGA GAGCCGCGTG GGAGACAGCA  
1201 AGACACACTC CAGTCAATTC CTGGCTAGGC AACATAATCA TGTTTGCCCC  
1251 CACACTGTGG GCGAGGATGA TACTGATGAC CCATTTCTTT AGCGTCCTCA  
1301 TAGCCAGGGA TCAGCTTGAA CAGGCTCTTA ACTGTGAGAT CTACGGAGCC  
1351 TGCTACTCCA TAGAACC ACT GGATCTACCT CCAATCATTC AAAGACTCCA  
1401 TGGCCTCAGC GCATTTTCAC TCCACAGTTA CTCTCCAGGT GAAATCAATA  
1451 GGGTGGCCGC ATGCCTCAGA AACTTGGGG TCCCGCCCTT GCGAGCTTGG  
1501 AGACACCGGG CCCGGAGCGT CCGCGCTAGG CTTCTGTCCA GAGGAGGCAG  
1551 GGCTGCCATA TGTGGCAAGT ACCTCTTCAA CTGGGCAGTA AGAACAAAGC  
1601 TCAAAC TCAC TCCAATAGCG GCCGCTGGCC GGCTGGACTT GTCCGGTTGG  
1651 TTCACGGCTG GCTACAGCGG GGGAGACATT TATCACAGCG TGTCTCATGC



1701 CCGGCCCCGC

## 5 HCV NS5B Truncation Mutant Polypeptide Sequence [SEQ ID NO:4]

1 SMSYSWTGAL VTPCAAEEQK LPINALSNSL LRHHNLVYST TSRSACQRQK  
 51 LRHHNLVYST TSRSACQRQK KVTFDRLQVL DSHYQDVLKE VKAAASKVKA  
 10 101 NLLSVEEACS LTPPHSAKSK FGYGAKDVRC HARKAVAHIN SVWKDLLEDS  
 151 VTPIDTTIMA KNEVFCVQPE KGGRKPARLI VFPDLGVRVC EKMALYDVVS  
 15 201 KLPLAVMGSS YGFQYSPGQR VEFLVQAWKS KKTPMGFSYD TRCFDSTVTE  
 251 SDIRTEEAIIY QCCDLDPQAR VAIKSLTERL YVGGPLTNSR GENCGYRRCR  
 301 ASGVLTTCSCG NTLTCYIKAR AACRAAGLQD CTMLVCGDDL VVICESAGVQ  
 20 351 EDAASLRAFT EAMTRYSAAPP GDPPQPEYDL ELITSCSSNV SVAHDGAGKR  
 401 VYYLTRDPTT PLARAAWETA RHTPVNSWLG NIIMFAPTLW ARMILMTHFF  
 25 451 SVLIARDQLE QALNCEIYGA CYSIEPLDLP PIIQRLHGLS AFSLHSYSPG  
 501 EINRVAACLR KLGVPPLRAW RHRARSVRAR LLSRGGRAAI CGKYLFWNAV  
 551 RTKLKLTPIA AAGRLDLGW FTAGYSGGDI YHSVSHARPR  
 30

Like other positive strand RNA viruses, HCV encodes an RNA-dependent RNA polymerase contained within the NS5B region. *See, e.g., Beherns, et al., EMBO J.* 15: 12-22 (1996); Hwang, *et al., Virology* 227: 439-446 (1997); Yuan, *et al., Biochem. Biophys. Res. Comm.* 232: 231-235 (1997). HCV NS5B catalyzes  
 35 phosphodiester bond formation resulting in new RNA molecules which are then packaged into progeny virions. Like the RNA-dependent polymerases of other positive strand RNA viruses, HCV NS5B is a membrane-associated protein Hwang, *et al., supra*. Expression of the full-length NS5B region in recombinant systems, baculovirus or *E. coli*, for example, results in a protein which is  
 40 membrane-associated or insoluble. *See, e.g., Beherns, et al., supra; Hwang, et al., supra; Yuan, et al., supra*. Although the overall amino acid composition of HCV NS5B is not hydrophobic, there is a 21-amino acid residue hydrophobic tail that could potentially serve as a membrane anchor region. This hydrophobic tail

is found in other genotypes of HCV (see Table 4), as well as other members of the *Flaviviridae* family, such as the pestiviruses (e.g., bovine viral diarrhea virus and classic swine fever virus).

5 Table 3. Alignment of the carboxy-terminus of NS5B sequences from various HCV genotypes.

		3001		3050
	Hcv_2c	RLDLSSWFT VSAGGGDIYH SVSRARPLL	<u>LLGLLLLCVG</u>	<u>VGIFLLPAR.</u>
10	(SEQ ID NO:5)			
	Hcv_J6	RLDLSSWFT VGAGGGDIYH SVSRARPLL	LLGLLLLFVG	VGLFLLPAR.
	(SEQ ID NO:6)			
15	Hcv_J8	SRLDLGWFT VGAGGGDIYH SVSHARPLL	LLCLLLLSVG	VGIFLLPAR.
	(SEQ ID NO:7)			
	Hcv_H	GRDLSGWFT AGYSGGDIYH SVSHARPRWF	WFCLLLLAAG	VGIYLLPNR.
20	(SEQ ID NO:8)			
	Hcv_Rice	GRDLSGWFT AGYSGGDIYH SVSHARPRWF	WFCLLLLAAG	VGIYLLPNR.
	(SEQ ID NO:9)			
	Hcv_1	GQLDLGWFT AGYSGGDIYH SVSHARPRWI	WFCLLLLAAG	VGIYLLPNR.
25	(SEQ ID NO:10)			
	Hcv_J1	GRDLSGWFT AGYSGGDIYH SVSHARPRWF	WFCLLLLAAG	VGIYLLPNR.
	(SEQ ID NO:11)			
30	Hcv_K1r3	SQLDLSSWFV AGYSGGDIYH SLSRARPRWF	MWCLLLLSVG	VGIYLLPNR.
	(SEQ ID NO:12)			
	Hcv_K1s3	SQLDLSSWFV AGYSGGDIYH SLSRARPRWF	MWCLLLLSVG	VGIYLLPNR.
	(SEQ ID NO:13)			
35	Hcv_K1r1	SQLDLSNWFV AGYSGGDVYH SLSRARPRWF	MLCLLLLSVG	VGIYLLPNR.
	(SEQ ID NO:14)			
	Hcv_K1s1	SQLDLSNWFV AGYSGGDVYH SLSRARPRWF	MLCLLLLSVG	VGIYLLPNR.
40	(SEQ ID NO:15)			
	Hcv_T	SQLDLKWFV AGYGGGDIYH SLSRARPRWF	MLCLLLLSVG	VGIYLLPNR*
	(SEQ ID NO:16)			
45	Hcv_Bk	SRLDLGWFT AGYSGGDIYH SLSRARPRWF	MLCLLLLSVG	VGIYLLPNR*
	(SEQ ID NO:17)			
	Hcv_Hb	SRLDLGWFT AGYSGGDIYH SLSRARPRWF	MLCLLLLSVG	VGIYLLPNR.
	(SEQ ID NO:18)			
50	Hcv_J483	SQLDLGWFT AGYSGGDIYH SLSRARPRWF	LLCLLLLSVG	VGIYLLPNR.

- (SEQ ID NO:19)
- Hcv\_C491 SQLDLSGWFV AGYSGGDIYH SLSRARPRWF PLCLLLLFVG VGIYLLPNR\*  
(SEQ ID NO:20)
- 5 Hcv\_C SQLDLSGWFV AGYNGGDIYH SLSRARPRWF MLCLLLLSVG VGIYLLPNR\*  
(SEQ ID NO:21)
- 10 Hcv\_C2 SRLDLSGWFV AGYGGGDIYH SLSRARPRWF MLCLLLLSVG VGIYLLPNR\*  
(SEQ ID NO:22)
- Hcv\_K1r2 SQLDLSGWFV AGYSGGDIYH SVSRARPRWF MWCLLLLSVG VGIYLLPNR.  
(SEQ ID NO:23)
- 15 Hcv\_K1s2 SQLDLSGWFV AGYSGGDIYH SVSRARPRWF MWCLLLLSVG VGIYLLPNR.  
(SEQ ID NO:24)
- Hcv\_Jt SQLDLSSWFV AGYSGGDIYH SLSRARPRWF MWCLLLLSVG VGIYLLPNR.  
(SEQ ID NO:25)
- 20 Hcv\_Pp SQLDLSGWFV AGYSGGDIYH SLSRARPRWF MWCLLLLSVG VGIYLLPNR.  
(SEQ ID NO:26)
- Hcv\_Jk1 SQLDLSGWFV AGYSGGDIYH SLSRARPRWF MWCLLLLSVG VGIYLLPNR\*  
(SEQ ID NO:27)
- 25 Hcv\_L1 SRLDLSGWFV AGYSGGDIYH SLSRARPRWF MLCLLLLSVG VGIYLLPNR\*  
(SEQ ID NO:28)
- 30 Hcv\_L2 SRLDLSSWFV AGYSGGDIYH SVSHARPRWF MLCLLLLSVG VGIYLLPNR\*  
(SEQ ID NO:29)
- Hcv\_N SQLDLSGWFV AGYSGGDIYH SLSRARPRWF MLCLLLLSVG VGIYLLPNR\*  
(SEQ ID NO:30)
- 35 Hcv\_3a GQLDLSSWFT VGVGGNDIYH SVSRARTRYL LLCLLLLTVG VGIFLLPAR.  
(SEQ ID NO:31)
- Hcv\_3b GQLDLSSWFT VGVGGNDIYH SVSRARTRHL LLCLLLLTVG VGIFLLPAR.  
(SEQ ID NO:32)
- 40 BVDV(NADL) LQGHYEQLQ LRTETNPVMG VGTERYKLGP IVNLLLRRLK ILLMTAVGVSS  
(SEQ ID NO:33)
- 45 CSFV GRHYEEL VLARKQFNMF QGTDRYNLGP IVNMVLRRLR VMMMTLIGRGV  
(SEQ ID NO:34)

Deletion of this hydrophobic tail of HCV NS5B releases the protein into the soluble portion of the cell, allowing for a greater recovery of soluble protein for screening for inhibitors of NS5B enzymatic activity. Additionally, soluble protein produced in this method would allow for determination of the structure of the protein via x-ray crystallography or other methods that are well known in the

art. This information could be used to discover or to guide the development of inhibitors. These inhibitors of NS5B potentially could have antiviral activity and, thus, could be used as therapeutic agents for the treatment of viruses of the *Flaviviridae* family, particularly HCV; flaviviruses such as yellow fever virus; 5 Dengue virus types 1-4; and pestiviruses, such as bovine viral diarrhea virus and classic swine fever, among others.

### Polypeptides

The polypeptides of the invention include the polypeptide of Table 3 [SEQ 10 ID NO:4] (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those which have the biological activity of HCV NS5B, and also those which have at least 70% identity to the polypeptide of Table 3 [SEQ ID NO:4] or the relevant portion, preferably at least 80% identity to the polypeptide of Table 3 [SEQ ID NO:4], and more preferably at least 90% 15 similarity (more preferably at least 90% identity) to the polypeptide of Table 3 [SEQ ID NO:4] and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of Table 3 [SEQ ID NO:4] and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and, more preferably, at least 50 20 amino acids.

A fragment is a variant polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned polypeptides. As with HCV NS5B polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a 25 part or region, most preferably as a single continuous region, a single larger polypeptide.

Preferred fragments include, for example, variants of the amino acid sequence of Table 3 [SEQ ID NO:4], thereof, such as a continuous series of residues that includes the amino terminus, or a continuous series of residues that 30 includes the carboxyl terminus. Degradation forms of the polypeptides of the invention in a host cell are also preferred. Further preferred are fragments characterized by structural or functional attributes, such as fragments that

comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding  
5 region, and high antigenic index regions.

Also preferred are biologically active fragments, which are those fragments that mediate activities of HCV NS5B, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal,  
10 especially in a human. Particularly preferred are fragments comprising receptors or domains of enzymes that confer a function essential for viability of HCV or the ability to initiate, or maintain cause disease in an individual, particularly a human.

Variants that are fragments of the polypeptides of the invention may be  
15 employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

### **Polynucleotides**

20 Another aspect of the invention relates to isolated polynucleotides, including the full length gene, that encode the HCV NS5B polypeptide having the deduced amino acid sequence of Table 3 [SEQ ID NO:3] and polynucleotides closely related thereto and variants thereof.

Using the information provided herein, a polynucleotide of the invention  
25 encoding the HCV NS5B polypeptide set forth in Table 3 [SEQ ID NO:3], may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using HCV NS5B cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention encoding the polypeptide  
30 sequence given in Table 3 [SEQ ID NO:4], typically a library of clones of chromosomal DNA of HCV NS5B in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a

partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent conditions. By sequencing the individual clones thus identified with sequencing primers designed from the original sequence, it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently, such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook, *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (See, in particular, Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Illustrative of the invention, the polynucleotide encoding the polypeptide set out in Table 3 [SEQ ID NO:4] was discovered in a DNA library derived from HCV NS5B.

The DNA sequence of HCV NS5B contains an open reading frame encoding a protein having about the number of amino acid residues set forth in Table 2 [SEQ ID NO: 2] with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known in the art. The polynucleotide of SEQ ID NO: 1 encodes the polypeptide of SEQ ID NO: 2.

The invention provides a polynucleotide sequence identical over its entire length to a sequence encoding the sequence in Table 3 [SEQ ID NO:3]. Also provided by this invention is the coding sequence for the mature polypeptide or a fragment thereof, by itself as well as the coding sequence for the mature polypeptide or a fragment in reading frame with other coding sequence, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence. The polynucleotide may also contain non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence which encode additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz, *et*

*al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA tag (Wilson, *et al.*, *Cell* 37: 767 (1984)). Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

5           A preferred embodiment of the invention is the polynucleotide set forth in SEQ ID NO:3 of Table 3, which encodes a HCV NS5B polypeptide.

          The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a viral polypeptide and more particularly a polypeptide  
10       of HCV NS5B having the amino acid sequence set out in Table 3 [SEQ ID NO:4]. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or an insertion sequence or editing) together with additional regions, that also may contain coding and/or non-coding sequences.

15           The invention further relates to variants of the polynucleotides described herein that encode for variants of the polypeptide having the deduced amino acid sequence of Table 3 [SEQ ID NO:4]. Variants that are fragments of the polynucleotides of the invention may be used to synthesize full-length polynucleotides of the invention.

20           Further particularly preferred embodiments are polynucleotides encoding HCV NS5B variants, that have the amino acid sequence of HCV NS5B polypeptide of Table 3 [SEQ ID NO:4] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions  
25       and deletions, that do not alter the properties and activities of HCV NS5B.

          Further preferred embodiments of the invention are polynucleotides that are at least 70% identical over their entire length to a polynucleotide encoding HCV NS5B polypeptide having the amino acid sequence set out in Table 3 [SEQ ID NO:4], and polynucleotides that are complementary to such polynucleotides.  
30       Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding HCV NS5B polypeptide and polynucleotides complementary thereto. In this

regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by the DNA of Table 3 [SEQ ID NO:3].

The invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein.

The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence encoding the polypeptide sequence set forth in SEQ ID NO:4 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence or a fragment thereof; and isolating said DNA sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers described elsewhere herein.



As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding HCV NS5B and to isolate  
5 cDNA and genomic clones of other genes that have a high sequence similarity to the HCV NS5B gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

10 For example, the coding region of the truncated HCV NS5B gene may be isolated by screening using the DNA sequence provided in SEQ ID NO:3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the  
15 probe hybridizes to.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays.

20 Polynucleotides of the invention that are oligonucleotides derived from the sequences of SEQ ID NOs:3 and/or 4 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in  
25 diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that may encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may  
30 play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As

generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When  
5 prosequences are removed, such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein),  
10 a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

15

#### **Vectors, host cells, expression**

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with  
20 vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the  
25 invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, BASIC METHODS IN MOLECULAR BIOLOGY, (1986) and Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as calcium  
30 phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as *streptococci*, *staphylococci*, *enterococci*, *E. coli*, *streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, hepatic cells, and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook, *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin

chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

5

### Diagnostic Assays

This invention is also related to the use of the HCV NS5B polynucleotides of the invention for use as diagnostic reagents. Detection of HCV NS5B in a eukaryote, particularly a mammal, and especially a human, will provide a  
10 diagnostic method for diagnosis of a disease. Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the HCV NS5B gene may be detected at the nucleic acid level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a bodily sample of an  
15 infected individual. Such a bodily sample can be either cells and tissues, such as bone, blood, muscle, cartilage, and skin. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification technique prior to analysis. RNA or cDNA may also be used in the same ways. Using amplification, characterization of the species and strain of prokaryote  
20 present in an individual, may be made by an analysis of the genotype of the prokaryote gene. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing amplified DNA to labeled HCV NS5B polynucleotide sequences. Perfectly matched sequences can be  
25 distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. *See, e.g., Myers, et al., Science, 230: 1242 (1985).* Sequence changes at specific locations also may be  
30 revealed by nuclease protection assays, such as RNase and S1 protection or a chemical cleavage method. *See, e.g., Cotton, et al., Proc. Natl. Acad. Sci., USA, 85: 4397-4401 (1985).*

Cells carrying mutations or polymorphisms in the gene of the invention may also be detected at the DNA level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations. It is particularly preferred to use RT-PCR in conjunction with automated  
5 detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to a nucleic acid encoding HCV NS5B can be used to identify and analyze mutations.

The invention further provides a process for diagnosing disease, preferably  
10 viral infections linked to the *Flaviviridae* family, particularly HCV; flaviviruses such as yellow fever virus; Dengue virus types 1-4; and pestiviruses, such as bovine viral diarrhea virus and classic swine fever, among others, comprising determining from a sample derived from an individual a increased level of expression of HCV NS5B polynucleotide having the sequence of Table 3 [SEQ  
15 ID NO:3]. Increased or decreased expression of HCV NS5B polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for  
20 detecting over-expression of HCV NS5B protein compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of an HCV NS5B protein, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western  
25 Blot analysis and ELISA assays.

### Antibodies

The polypeptides of the invention or variants thereof, or cells expressing them can be used as an immunogen to produce antibodies immunospecific for  
30 such polypeptides. "Antibodies" as used herein includes monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and

humanized antibodies, as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

Antibodies generated against the polypeptides of the invention can be obtained by administering the polypeptides or epitope-bearing fragments, analogues or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, *et al.*, *Nature* 256: 495-497 (1975); Kozbor, *et al.*, *Immunology Today* 4: 72 (1983); Cole, *et al.*, pg. 77-96 in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-HCV NS5B or from naive libraries (McCafferty, *et al.*, (1990), *Nature* 348: 552-554; Marks, *et al.*, (1992) *Biotechnology* 10: 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, *et al.*, (1991) *Nature* 352: 624-628).

If two antigen binding domains are present each domain may be directed against a different epitope - termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides to purify the polypeptides by affinity chromatography.

Thus, among others, antibodies against HCV NS5B polypeptide may be employed to treat viral infections, preferably viruses of the *Flaviviridae* family, particularly HCV; flaviviruses such as yellow fever virus; Dengue virus types 1-4; and pestiviruses, such as bovine viral diarrhea virus, and classic swine fever, among others.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants that form a particular aspect of this invention. The term "antigenically equivalent derivative" as used herein encompasses a polypeptide or its equivalent which will be specifically recognized  
5 by certain antibodies which, when raised to the protein or polypeptide according to the invention, interfere with the immediate physical interaction between pathogen and mammalian host. The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act  
10 to interfere with the immediate physical interaction between pathogen and mammalian host.

The polypeptide, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability  
15 to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple copies of the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to  
20 improve immunogenicity so as to obviate the use of a carrier.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized"; where the complementarity determining region(s) of the hybridoma-derived antibody has been transplanted  
25 into a human monoclonal antibody, for example as described in Jones, *et al.*, *Nature* 321: 522-525 (1986), or Tempest, *et al.*, *Biotechnology* 9: 266-273 (1991).

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid  
30 DNA into muscles (Wolff, *et al.*, *Hum. Mol. Genet.* 1: 363 (1992), Manthorpe, *et al.*, *Hum. Gene Ther.* 4: 419 (1993)), delivery of DNA complexed with specific protein carriers (Wu, *et al.*, *J. Biol. Chem.* 264: 16985 (1989)), coprecipitation of

- DNA with calcium phosphate (Benvenisty & Reshef, *Proc. Natl. Acad. Sci. USA*, 83: 9551 (1986)), encapsulation of DNA in various forms of liposomes (Kameda, *et al.*, *Science* 243: 375 (1989)), particle bombardment (Tang, *et al.*, *Nature* 356: 152 (1992), Eisenbraun, *et al.*, *DNA Cell Biol.* 12:791 (1993)) and *in vivo*
- 5 infection using cloned retroviral vectors (Seeger, *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 5849 (1984)).

### Antagonists and agonists - assays and molecules

Polypeptides of the invention may also be used to assess the binding of

10 small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan, *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

15 The present invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of HCV NS5B polypeptides or polynucleotides, particularly those compounds that are virustatic. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a viral

20 fragment or component, or a preparation of any thereof, comprising HCV NS5B polypeptide (SEQ ID NO:4) and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be an HCV NS5B agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the HCV NS5B polypeptide (SEQ ID NO:4) is reflected in

25 decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of HCV NS5B polypeptide are most likely to be good antagonists. Molecules that bind well and increase the rate of product production from substrate are agonists. Detection of the rate or level of production of product from substrate may be

30 enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric labeled substrate converted into



product, a reporter gene that is responsive to changes in HCV NS5B polynucleotide or polypeptide activity, and binding assays known in the art

Another example of an assay for HCV NS5B antagonists is a competitive assay that combines HCV NS5B (SEQ ID NO:4) and a potential antagonist with  
5 HCV NS5B-binding molecules, recombinant HCV NS5B binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. HCV NS5B (SEQ ID NO:4) can be labeled, such as by radioactivity or a colorimetric compound, such that the  
10 number of HCV NS5B molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide or polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also  
15 may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing HCV NS5B-induced activities, thereby preventing the action of HCV NS5B by excluding HCV NS5B from binding.

Potential antagonists include a small molecule that binds to and occupies  
20 the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include, but are not limited to, small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules. *See Okano, J. Neurochem. 56: 560 (1991); OLIGODEOXYNUCLEOTIDES AS*  
25 *ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules. Preferred potential antagonists include compounds related to and variants of HCV NS5B.

The DNA sequences encoding the HCV NS5B polypeptides provided herein may be used in the discovery and development of antibacterial compounds.  
30 The encoded protein, upon expression, can be used as a target for the screening of antiviral drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating

sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide or inhibitor of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of virus particles, to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block HCV NS5B protein-mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian tyrosine kinases (Rosenshine, *et al.*, *Infect. Immun.* 60: 2211 (1992); to block viral adhesion between mammalian extracellular matrix proteins and viral HCV NS5B proteins that mediate tissue damage and; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

The antagonists and agonists of the invention may be employed, for instance, to inhibit and treat viruses linked to the *Flaviviridae* family, particularly HCV; flaviviruses such as yellow fever virus; Dengue virus types 1-4; and pestiviruses, such as bovine viral diarrhea virus and classic swine fever, among others.

### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with HCV NS5B (SEQ ID NO:4), or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly viral infection and, most particularly, HCV infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector to direct expression of HCV NS5B, or a fragment or a variant thereof, for expressing

HCV NS5B, or a fragment or a variant thereof in vivo in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that disease is already established  
5 within the individual or not. One way of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid.

A further aspect of the invention relates to an immunological composition  
10 which, when introduced into an individual capable or having induced within it an immunological response, induces an immunological response in such individual to HCV NS5B, wherein the composition comprises a recombinant HCV NS5B or protein coded therefrom comprising DNA which codes for and expresses an antigen of said HCV NS5B or protein coded therefrom. The immunological  
15 response may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

An HCV NS5B polypeptide or a fragment thereof may be fused with co-protein which may not by itself produce antibodies, but is capable of stabilizing  
20 the first protein and producing a fused protein which will have immunogenic and protective properties. Thus, fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from Hemophilus influenzae, Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilize the protein and facilitate production and purification  
25 thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides or polynucleotides of the  
30 invention and immunostimulatory DNA sequences, such as those described in Sato, *et al. Science* 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof which have been shown to encode non-variable regions of bacterial cell surface proteins in DNA constructs used in such genetic immunization experiments in animal models of infection with HCV

5 will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic treatments of

10 bacterial infection, particularly HCV infection, in mammals, particularly humans.

The polypeptide may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of viruses, for example by blocking adherence of viruses to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused, *e.g.*, by mechanical, chemical

15 or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant protein of the invention together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably

20 administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the bodily fluid, preferably the blood,

25 of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may

30 also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The

dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation

While the invention has been described with reference to HCV NS5B protein, it is to be understood that fragments of the naturally occurring protein and  
5 similar proteins with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant protein are contemplated within the scope of the invention.

### Compositions, kits and administration

10 The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or their agonists or antagonists. The polypeptides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions  
15 comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates  
20 to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

25 The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the pharmaceutical composition may be  
30 administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively, the composition may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially HCV wound infections.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The polypeptides or other

compounds of this invention will preferably be present at a concentration of 1 mg/ml to 10 mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit  
5 dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

10

## EXAMPLES / BIOLOGICAL METHODS

### Example 1: Preparation of HCV NS5B truncation mutant

The HCV NS5B region (SEQ ID NO:2) was cloned into a bacterial expression vector pET-15b (Novagen) such that an initiating methionine and  
15 hexahistidine tag was added to the amino terminus of the protein to generate pLG65. Two additional plasmids were made in which either the 21 carboxy-terminal amino acids of HCV NS5B were deleted or replaced by a Flag epitope tag (Hopp, *et al.*, *Biotechnology* 6: 1205-1210 (1988)), which is a hydrophilic stretch of amino acids. NS5B expression constructs were transferred into the E.  
20 coli strain BL21(DE3), and the NS5B proteins were induced by addition of IPTG using standard methods. Bacteria were harvested, and lysed by sonication three times for 30 seconds in extraction buffer (20 mM Tris, pH 7.5, 20% glycerol, 200 mM NaCl, 1 mM EDTA, 10 mM dithiothreitol, 10 mgs/ml lysozyme, and a protease inhibitor cocktail (COMPLETE tabs from Behringer Mannheim).

25

### Example 2: HCV NS5B truncation mutant has improved solubility over full-length HCV NS5B protein

Some of the material generated in Example 1 was reserved as a sample of the total protein (T). The sample was centrifuged at 14,000 rpm at 4°C in a  
30 Eppendorf 5415C microfuge for fifteen minutes. The supernatant (S1) was removed and spun for 30 minutes at 4°C at 100,000 x g. The supernatant (S2) from this spin represented the truly soluble material. Equal amounts of protein

from were analyzed for each clone from each sample (T. S1. S2) were analyzed by SDS-containing polyacrylamide gel electrophoresis, followed by western blotting using standard procedures. HCV NS5B was detected using a rabbit polyclonal antisera which had been generated using a peptide derived from the NS5B region (amino acids 385-403 of HCV NS5B (SEQ ID NO:2)) coupled to keyhole limpet hemocyanin (KLH). Results show that the truncated HCV NS5B proteins in which the carboxy terminal 21 amino acids were either deleted or replaced by the Flag epitope tag sequence were present in the soluble fraction (S2) in far greater amounts when compared with the full length NS5B protein which was either barely detectable or absent in the soluble fraction (S2).

All publications including, but not limited to, patents and patent applications, cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention, including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore, the examples provided herein are to be construed as merely illustrative and are not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.



## What is claimed is:

1. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
  - 5 (a) a polynucleotide having at least a 95% identity to a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:4;
  - (b) a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 95% identical to the amino acid sequence of SEQ ID NO:4; and
  - 10 (c) a polynucleotide which is complementary to the polynucleotide(s) of (a) or (b).
2. The polynucleotide according to Claim 1 wherein the polynucleotide is DNA.
- 15 3. The polynucleotide according to Claim 1 wherein the polynucleotide is RNA.
4. An isolated polynucleotide comprising the nucleic acid sequence set forth in SEQ ID NO:3.
- 20 5. The polynucleotide according to Claim 4 comprising nucleotide 11792 to 13562 set forth in SEQ ID NO:3.
- 25 6. An isolated polynucleotide which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:4.
7. A vector comprising the polynucleotide according to Claim 1.
- 30 8. A host cell comprising the vector according to Claim 7.
9. A process for producing a polypeptide, which process comprises: expressing from the host cell according to Claim 8 a polypeptide or a fragment encoded by the polynucleotide sequence comprising SEQ ID NO:3 under conditions sufficient for  
35 the production of said polypeptide or fragment.
10. A polypeptide comprising an amino acid sequence which is at least 95% identical to the amino acid sequence according to SEQ ID NO:4.

11. A polypeptide comprising an amino acid sequence as set forth in SEQ  
ID NO:4.

12. An antibody against the polypeptide according to Claim 10.

5

13. An antagonist which inhibits the activity or expression of the  
polypeptide according to Claim 10.

14. A method for identifying compounds which inhibit or which activate an  
10 the polypeptide according to Claim 10, which method comprises:

a) contacting a composition comprising the polypeptide with a candidate  
compound to be screened under conditions to permit interaction between the compound  
and the polypeptide; and

b) determining whether the compound interacts with, and activates, or inhibits  
15 the activity of the polypeptide.

15. The method according to Claim 14, wherein the interaction in the contacting  
step of (a) is associated with a second component capable of providing a detectable  
signal in response to the interaction of the polypeptide with the compound and wherein  
20 the determining step of (b) detects the presence or absence of a signal generated from the  
interaction of the compound with the polypeptide.

16. A method for inducing an immunological response in a mammal which  
method comprises inoculating the mammal with the HCV NS5B polypeptide of Claim  
25 10, or a fragment or variant thereof, in an effective amount sufficient to produce an  
antibody and/or T cell immune response in said mammal.

17. A method of inducing immunological response in a mammal in need  
thereof, which method comprises delivering a nucleic acid vector, wherein said vector  
30 directs expression of the HCV NS5B polypeptide according to Claim 10 or which vector  
expresses a fragment or a variant of the HCV NS5B polypeptide *in vivo* in order to  
induce an immunological response sufficient to produce an antibody and/ or T cell  
immune response in said mammal.

18. A method of protecting a mammal from a disease caused by viruses of  
the *Flaviviridae* family, which method comprises administering to said mammal a

therapeutically effective amount of an antibody produced against the polypeptide of Claim 10.

## SEQUENCE LISTING

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<120> HEPATITIS C VIRUS NS5B TRUNCATED PROTEIN  
AND METHODS THEREOF TO IDENTIFY ANTIVIRAL COMPOUNDS

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SUBSTITUTE SHEET (RULE 26)

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SUBSTITUTE SHEET (RULE 26)

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Ser Arg Gly Gly Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp  
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 Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Pro Ala Ala Gly Arg  
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 Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile  
 545 550 555 560  
 Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Phe Trp Phe Cys Leu  
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&lt;210&gt; 3

&lt;211&gt; 1710

&lt;212&gt; DNA

&lt;213&gt; Viral

&lt;400&gt; 3

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SUBSTITUTE SHEET (RULE 26)

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&lt;211&gt; 590

&lt;212&gt; PRT

&lt;213&gt; Viral

&lt;400&gt; 4

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 35 40 45  
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 85 90 95  
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 145 150 155 160  
 Lys Asn Glu Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro  
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 Val Gln Ala Trp Lys Ser Lys Lys Thr Pro Met Gly Phe Ser Tyr Asp  
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 Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr Glu  
 245 250 255

SUBSTITUTE SHEET (RULE 26)



Glu Ala Ile Tyr Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg Val Ala  
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 Ser Arg Gly Glu Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val  
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 Ala Ala Cys Arg Ala Ala Gly Leu Gln Asp Cys Thr Met Leu Val Cys  
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 Gly Asp Asp Leu Val Val Ile Cys Glu Ser Ala Gly Val Gln Glu Asp  
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 355 360 365  
 Pro Pro Gly Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr  
 370 375 380  
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 Pro Ile Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser  
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 580 585 590

SUBSTITUTE SHEET (RULE 26)

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<210> 6  
 <211> 49  
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 <213> Viral

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 Asp Ile Tyr His Ser Val Ser Arg Ala Arg Pro Arg Leu Leu Leu Leu  
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 Gly Leu Leu Leu Leu Phe Val Gly Val Gly Leu Phe Leu Leu Pro Ala  
 35 40 45  
 Arg

<210> 7  
 <211> 49  
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 <213> Viral

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 20 25 30

**SUBSTITUTE SHEET (RULE 26)**

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<210> 8  
 <211> 50  
 <212> PRT  
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 1 5 10 15  
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 35 40 45  
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<210> 9  
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 <212> PRT  
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 35 40 45  
 Arg

<210> 10  
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 <212> PRT  
 <213> Viral

<400> 10  
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 1 5 10 15

**SUBSTITUTE SHEET (RULE 26)**

Asp Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Ile Trp Phe  
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                   35                  40                  45  
 Arg

<210> 11  
 <211> 49  
 <212> PRT  
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<400> 11  
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 Asp Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Phe Trp Phe  
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 Cys Leu Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn  
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 Arg

<210> 12  
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 <213> Viral

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 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp  
                   20                  25                  30  
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 Arg

<210> 13  
 <211> 49  
 <212> PRT  
 <213> Viral

**SUBSTITUTE SHEET (RULE 26)**

&lt;400&gt; 13

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 1 5 10 15  
 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp  
 20 25 30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45  
 Arg

&lt;210&gt; 14

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Viral

&lt;400&gt; 14

Ser Gln Leu Asp Leu Ser Asn Trp Phe Val Ala Gly Tyr Ser Gly Gly  
 1 5 10 15  
 Asp Val Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
 20 25 30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45  
 Arg

&lt;210&gt; 15

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Viral

&lt;400&gt; 15

Ser Gln Leu Asp Leu Ser Asn Trp Phe Val Ala Gly Tyr Ser Gly Gly  
 1 5 10 15  
 Asp Val Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
 20 25 30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45  
 Arg

&lt;210&gt; 16

&lt;211&gt; 49

SUBSTITUTE SHEET (RULE 26)

&lt;212&gt; PRT

&lt;213&gt; Viral

&lt;400&gt; 16

Ser Gln Leu Asp Leu Ser Lys Trp Phe Val Ala Gly Tyr Gly Gly Gly  
 1                      5                      10                      15  
 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
                     20                      25                      30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
                     35                      40                      45  
 Arg

&lt;210&gt; 17

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Viral

&lt;400&gt; 17

Ser Arg Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly  
 1                      5                      10                      15  
 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
                     20                      25                      30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
                     35                      40                      45  
 Arg

&lt;210&gt; 18

&lt;211&gt; 49

&lt;212&gt; PRT

&lt;213&gt; Viral

&lt;400&gt; 18

Ser Arg Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly  
 1                      5                      10                      15  
 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
                     20                      25                      30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
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 Arg

SUBSTITUTE SHEET (RULE 26)

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 35 40 45  
 Arg

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 <212> PRT  
 <213> Viral

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 <211> 49  
 <212> PRT  
 <213> Viral

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 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
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 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45  
 Arg

**SUBSTITUTE SHEET (RULE 26)**

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 <212> PRT  
 <213> Viral

<400> 22

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Asp	Ile	Tyr	His	Ser	Leu	Ser	Arg	Ala	Arg	Pro	Arg	Trp	Phe	Met	Leu
			20					25					30		
Cys	Leu	Leu	Leu	Leu	Ser	Val	Gly	Val	Gly	Ile	Tyr	Leu	Leu	Pro	Asn
			35				40					45			
Arg															

<210> 23  
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 <212> PRT  
 <213> Viral

<400> 23

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1				5					10					15	
Asp	Ile	Tyr	His	Ser	Val	Ser	Arg	Ala	Arg	Pro	Arg	Trp	Phe	Met	Trp
			20					25					30		
Cys	Leu	Leu	Leu	Leu	Ser	Val	Gly	Val	Gly	Ile	Tyr	Leu	Leu	Pro	Asn
			35				40					45			
Arg															

<210> 24  
 <211> 49  
 <212> PRT  
 <213> Viral

<400> 24

Ser	Gln	Leu	Asp	Leu	Ser	Gly	Trp	Phe	Val	Ala	Gly	Tyr	Ser	Gly	Gly
1				5					10					15	
Asp	Ile	Tyr	His	Ser	Val	Ser	Arg	Ala	Arg	Pro	Arg	Trp	Phe	Met	Trp
			20					25					30		

**SUBSTITUTE SHEET (RULE 26)**



Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45

Arg

<210> 25  
 <211> 49  
 <212> PRT  
 <213> Viral

<400> 25

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 1 5 10 15  
 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp  
 20 25 30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45

Arg

<210> 26  
 <211> 49  
 <212> PRT  
 <213> Viral

<400> 26

Ser Gln Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly  
 1 5 10 15  
 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp  
 20 25 30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
 35 40 45

Arg

<210> 27  
 <211> 49  
 <212> PRT  
 <213> Viral

<400> 27

Ser Gln Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly  
 1 5 10 15

**SUBSTITUTE SHEET (RULE 26)**

Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp  
                   20                  25                  30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
                   35                  40                  45  
 Arg

<210> 28  
 <211> 49  
 <212> PRT  
 <213> Viral

<400> 28  
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 Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu  
                   20                  25                  30  
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                   35                  40                  45  
 Arg

<210> 29  
 <211> 49  
 <212> PRT  
 <213> Viral

<400> 29  
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 Asp Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Phe Met Leu  
                   20                  25                  30  
 Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn  
                   35                  40                  45  
 Arg

<210> 30  
 <211> 49  
 <212> PRT  
 <213> Viral

**SUBSTITUTE SHEET (RULE 26)**

<210> 31

<211> 49

<212> PRT

<213> Viral

<400> 31

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<210> 32

<211> 49

<212> PRT

<213> Viral

<400> 32

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<210> 33

<211> 51

<212> PRT

<213> Viral

<400> 33

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          20          25          30
Asn Leu Leu Leu Arg Arg Leu Lys Ile Leu Leu Met Thr Ala Val Gly
          35          40          45
Val Ser Ser
          50

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<210> 34

<211> 48

<212> PRT

<213> Viral

<400> 34

Gly	Arg	His	Tyr	Glu	Glu	Leu	Val	Leu	Ala	Arg	Lys	Gln	Phe	Asn	Asn
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Phe	Gln	Gly	Thr	Asp	Arg	Tyr	Asn	Leu	Gly	Pro	Ile	Val	Asn	Met	Val
			20					25					30		
Leu	Arg	Arg	Leu	Arg	Val	Met	Met	Met	Thr	Leu	Ile	Gly	Arg	Gly	Val
		35					40					45			

**SUBSTITUTE SHEET (RULE 26)**

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26070

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/11, 15/63, 1/21; A61K 48/00, 35/00, 38/00; C12Q 1/70

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/5, 69.1, 69.3, 252.3, 320.1; 514/2, 44; 424/93.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

SEQ ID NO: 3 and 4, APS, MEDLINE, WPIDS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	KOLYKHALOV, A.A. et al. Transmission of Hepatitis C by Intrahepatic Inoculation with Transcribed RNA. Science. 25 July 1997, Vol. 277, pages 570-574, see attached sequence alignments.	1-5, 7, 8, 17 --- 6, 9-16, 18
X - Y	YANAGI, M. et al. Transcripts From a Single Full-Length cDNA Clone of Hepatitis C Virus are Infectious when Directly Transfected into the Liver of a Chimpanzee. PNAS USA. August 1997, Vol. 94, pages 8738-8743, see attached sequence alignments.	1-5, 7, 8, 17 --- 6, 9-16, 18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 MARCH 1999

Date of mailing of the international search report

19 MAR 1999

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26070

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	INCHAUSPE, G. et al. Genomic Structure of the Human Prototype Strain H of Hepatitis C Virus: Comparison with American and Japanese Isolates. PNAS USA. November 1991, Vol. 88, pages 10292-10296, see attached sequence alignments.	1-5, 7, 8, 17 --- 6, 9-16, 18
X, E	US 5,861,267 A (SU) 19 January 1999, see col 3-7 and attached sequence alignment.	1-5, 7, 8, 17 --- 6, 9-16, 18

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26070

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

536/23.1; 435/5, 69.1, 69.3, 252.3, 320.1; 514/2, 44; 424/93.1

